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1: J Biotechnol. 2005 Jul 23; [Epub ahead of print]

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## Metalloproteinase activity is the sole factor responsible for the growth-promoting effect of conditioned medium in *Trichoplusia ni* insect cell cultures.

Eriksson U, Hassel J, Lullau E, Haggstrom L.

School of Biotechnology, Department of Bioprocess Technology, Royal Institute of Technology, AlbaNova University Center, SE-106 91 Stockholm, Sweden.

Conditioned medium (CM) taken from a serum-free culture of *Trichoplusia ni* (BTI-Tn-5B1-4, High Five) cells on days 2 and 3, shortened the lagphase and increased the maximum cell density when added to *T. ni* cultures with low-inoculum cell density. Gel filtration fractions of CM, eluting at around 45kDa, stimulated cell proliferation even better than CM. A protein in the gel filtration fraction was identified by N-terminal amino acid sequencing as a proteinase, related to a snake venom metalloproteinase. Casein zymography showed, multiple metalloproteinase bands between 48 and 25kDa, as well as precursor forms above 48kDa. Metalloproteinase bands below the main band at 48kDa were autocatalytic degradation products. Metalloproteinase activity was the sole factor responsible for the growth stimulating effect of CM as shown by using the specific metalloproteinase inhibitor dl-thiorphan. Metalloproteinases have recently been shown to release growth factors from sequestering extracellular proteins. We propose that the metalloproteinase is involved in autocrine regulation of *T. ni* proliferation in serum-free media. In addition, a gel filtration fraction of CM, eluting at about 10kDa, inhibited cell growth. Apart from a lysozyme precursor protein and a cyclophilin-like protein, a kazal-type proteinase inhibitor could be identified in this fraction.

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